Hybridization-based DNA capture and next generation sequencing of whole mitochondrial genome from old skeletal remain samples

Eun Young Lee · Hwan Young Lee · Kyoung-Jin Shin

Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

Introduction

Mitochondrial DNA (mtDNA) typing is very useful tool for identification of challenging samples in nuclear DNA analysis due to severely damage or low level of endogenous DNA. Especially, because the specimens obtained from old skeletal remains contain a large portion of contaminating exogenous DNA, an enrichment step is required to allow efficient target-specific sequencing analysis. Here, we demonstrated the hybridization-based capture and enrichment method which targets to mtDNA-specific fragments from old skeletal remain samples and applied to the Illumina platform for the NGS analysis. Moreover, we analyzed sequence variations in whole mitochondrial genome from NGS results of ten samples and assigned the relevant haplogroup to each sample.

Materials and Methods

DNA samples

A total ten DNA samples, a control DNA 2800M (Promega Corp., Madison, MI, USA) and nine DNAs from 50-year-old skeletal remains were tested in this study. The DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Quantifiler® Trio Kit (Applied Biosystems, Foster City, CA, USA). DNAs were sheared to obtain a fragment size at approximately 500 bp using a Covaris S2 Focused-ultrasonicator (Covaris, Inc., Woburn, MA, USA) and 200 ng of sheared DNA was subjected to prepare libraries.

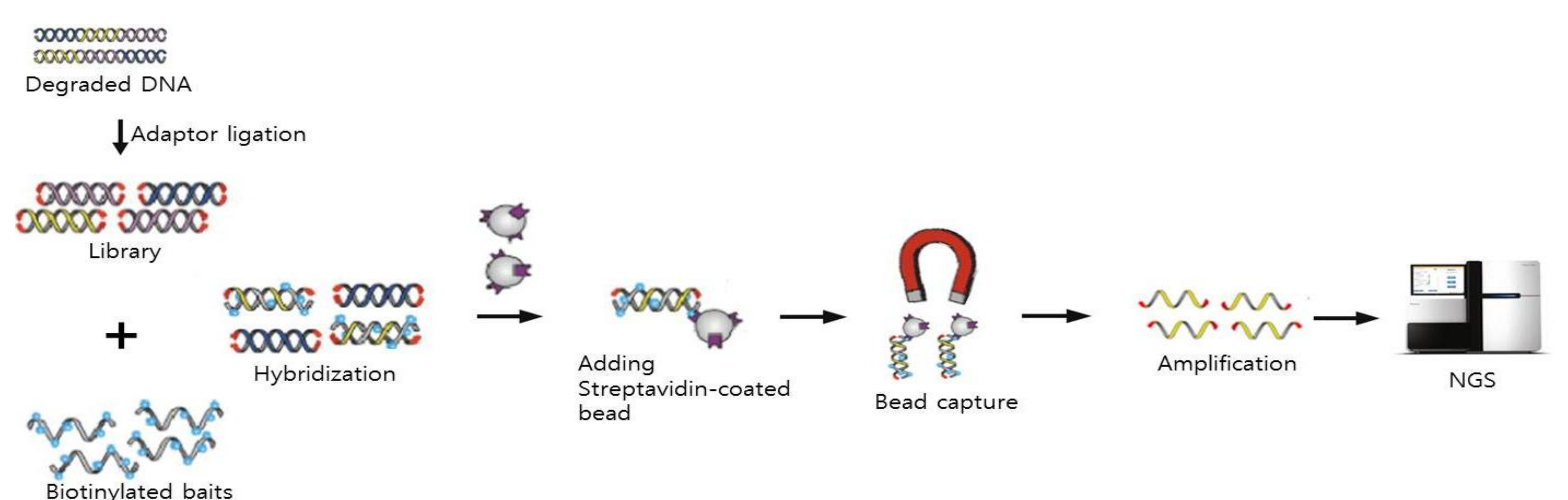
Target capture and library preparation

The RNA probes were designed to target the whole mitochondrial genome with 4× tiling density. The library preparation and hybridization-based target capture were performed according to the protocol of SureSelectXT Target Enrichment System (Agilent Technologies, Inc.) for Illumina sequencing platform (Fig. 1). Following PCR cleanup using Agencourt® AMPure® XP beads (Beckman Coulter, Inc., Indianapolis, IN, USA), libraries were quantified using KAPA library quantification kits (KAPA Biosystems, Inc., Wilmington, MA, USA), and electrophoresed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.)to assess fragment size and distribution.

Next generation sequencing and data analysis

NGS of captured libraries was performed on a MiSeq® system using a MiSeq Reagent Nano Kit v2 (500 cycles) (Illumina, Inc.). Sequence variations were analyzed using MToolBox (https://sourceforge.net/projects/mtoolbox) referring to rCRS. According to observed mutation motif, each sample was assigned to a certain haplogroup referring to PhyloTree Build 16 (http://www.phylotree.org).

Fig. 1. Workflow of library preparation, target enrichment and NGS



Results

Fig. 2. Observed reads per sample

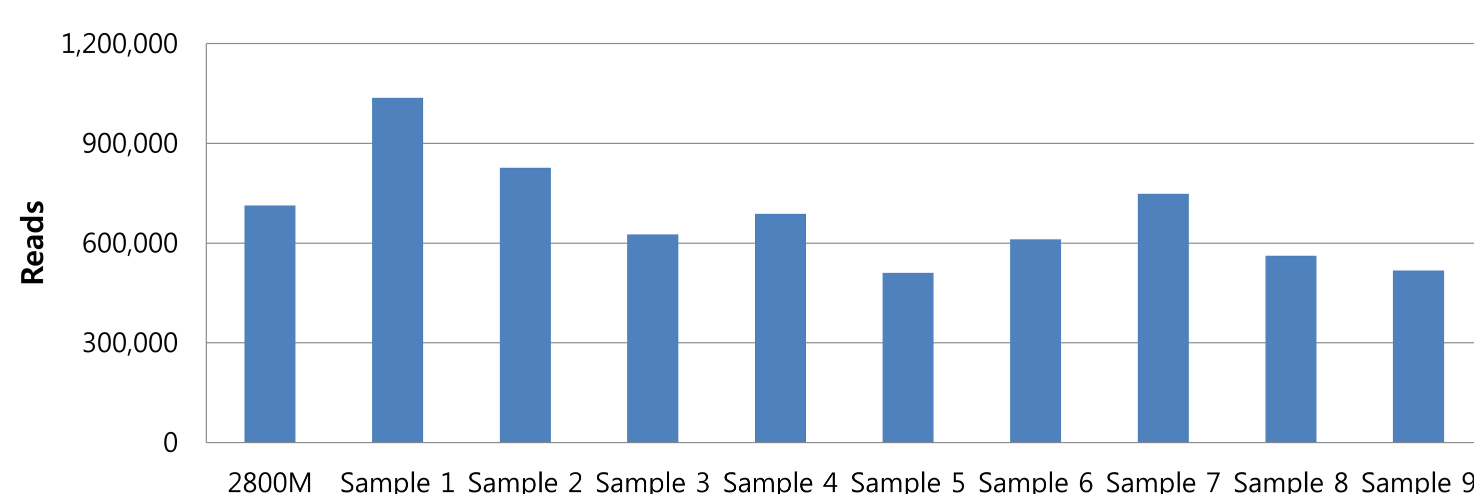


Fig. 3. Average depth and capture efficiency of mtDNA-specific reads

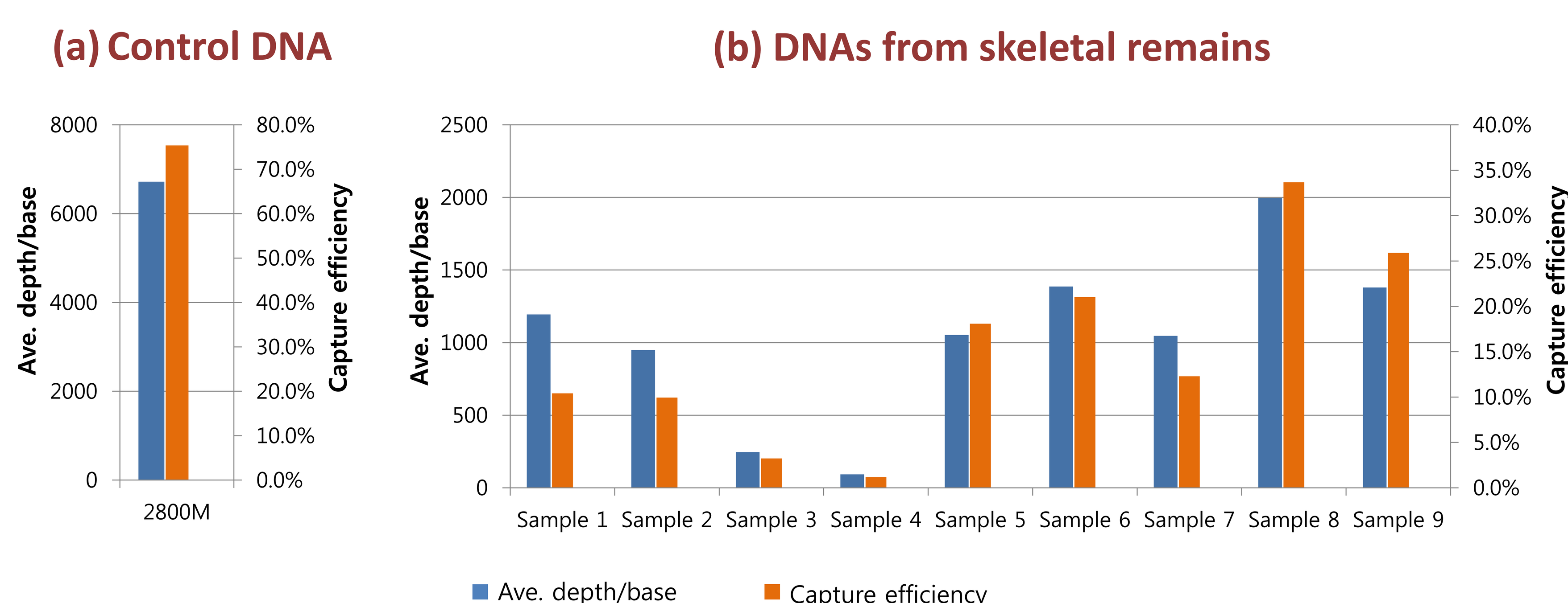


Fig. 4. Capture efficiency vs % of human DNA

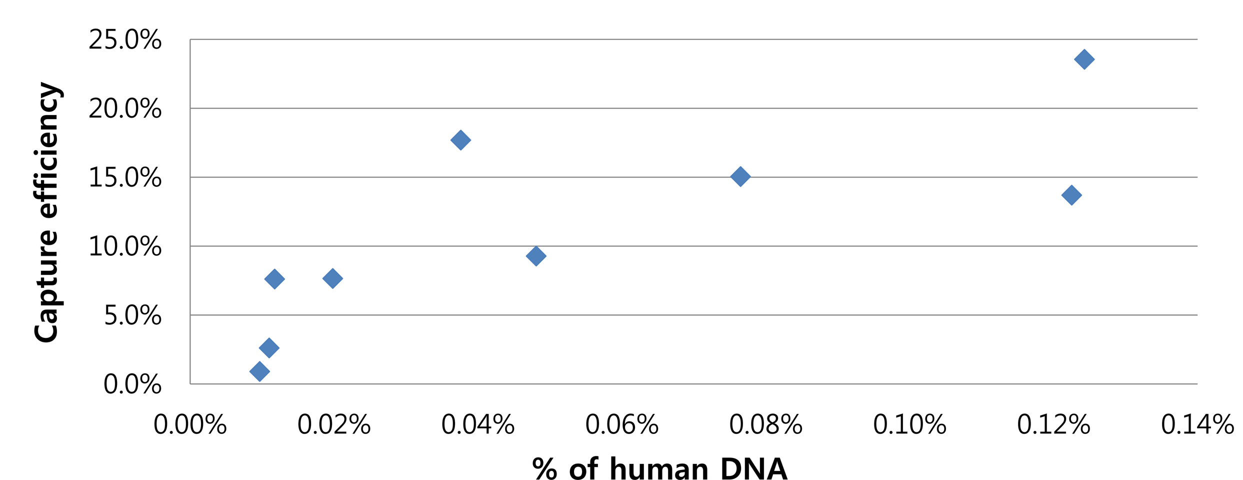


Table 1. Observed mutation motifs and assigned haplogroups

Sample ID	Variants	Haplogroup
2800M	152C 263G 315.1C 477C 750G 1438G 3010A 4769G 8860G 15326G 16519C	H1c4a
Sample 1	73G 263G 315.1C 489C 750G 1438G 2706G 3010A 4769G 4883T 5178A 7028T 7153C 8414T 8701G 8860G 9540C 10398G 10400T 10873C 11696A 11719A 12705T 14668T 14766T 14783C 15043A 15301A 15326G 16223T 16231C 16362C	D4j14
Sample 2	73G 152C 235G 263G 309.1C 315.1C 523d 524d 663G 750G 1438G 1736G 2706G 4248C 4769G 4824G 7028T 8794T 8860G 11719A 12705T 14766T 15258G 15326G 16093C 16223T 16290T 16319A 16362C	A2u1
Sample 3	73G 93G 210G 263G 309.1C 315.1C 523d 524d 709A 750G 1438G 2706G 3396C 3537G 4577T 4769G 7028T 8281-8289d 8584A 8860G 9950C 9962A 10398G 11149A 11151T 11719A 12234G 12570G 14149T 14766T 15235G 15326G 16129A 16140C 16187T 16189C 16266G 16519C	B5a2a1
…	…	…
Sample 9	73G 152C 249d 263G 309.1C 309.2C 315.1C 523d 524d 750G 1438G 2706G 3970T 4732G 4769G 5147A 6392C 6962A 7028T 8860G 9377G 10310A 10609C 10976T 11719A 12406A 12633T 12882T 13928C 13932T 14476A 14766T 15326G 15954G 16129A 16182C 16183C 16189C 16232A 16249C 16266T 16304C 16311C 16344T 16519C	F1b1a1a

Conclusions

- We demonstrated the hybridization-based capture and enrichment method which targets to mtDNA-specific fragments from old skeletal remain samples.
- The capture efficiency was affected by the ratio of human-originated DNA and that of skeletal remain specimens significantly declined compared to highly pure DNA sample.
- The whole mitochondrial genome sequences were identified and all samples were assigned to the relevant haplogroups according to their sequence variants.
- The method presented in our study will facilitate NGS analysis of whole mtDNA genome even from degraded DNA samples including high level of exogenous materials.

Acknowledgement

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